
PHYLOGENETIC RELATIONSHIPS
IN SUBTRIBE ASCLEPIADINAE
(APOCYNACEAE:
ASCLEPIADOIDEAE)¹

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ABSTRACT

A first approximation of evolutionary relationships within the Asclepiadinae (Apocynaceae: Asclepiadoideae) is inferred from two DNA data sets, from the nuclear ITS region, and from plastid *trnT-L* and *trnL-F* spacers and *trnL* intron. Both the subtribe as a whole and the phylogenetic radiation of largely herbaceous genera around *Asclepias* are monophyletic and well supported. While resolution within the *Asclepias* complex is limited, the lineages identified demonstrate that current generic delimitation is unsatisfactory. *Asclepias* should be either restricted to New World members of the subtribe or expanded to encompass the entire radiation. In the African species, it is shown that, although the corona is taxonomically important for recognizing species, it can be misleading as an indicator of phylogenetic relationship. Vegetative similarities often prove a more reliable guide, but more detailed studies are needed to refine these lineages and to detect morphological characters to aid in their practical recognition.

Key words: Apocynaceae, Asclepiadaceae, Asclepiadinae, phylogenetics, ITS, *trnT-L*, *trnL-F*, *trnL*.

Generic delimitation has long been problematic in the Asclepiadinae (Apocynaceae: Asclepiadoideae) sensu Liede (1997). Brown (1902: 299) commented in the *Flora of Tropical Africa*: “Undoubtedly *Xysmalobium* R. Br., *Asclepias* L. and *Schizoglossum* E. Mey. are but artificial divisions of one natural genus, since they cannot be separated by characters that do not break down at some point.” Nevertheless, he went on to assign species to the three genera on the basis of the form of the corona—cucullate in *Asclepias*, dorsally flattened in *Schizoglossum*, and thickened or laterally compressed in *Xysmalobium*. Such an artificial arrangement separated apparently allied species, such as *A. lisianthoides* (Decne.) N. E. Br., *S. carsonii* (N. E. Br.) N. E. Br., and *X. ceciliae* N. E. Br., all currently referred to *Glossostelma* Schltr. (Goyder, 1995). Subsequent morphological studies have proposed the use of non-coronal characters to reunite some of these natural groups. Bullock (1952, 1953a, b, 1955, 1956, 1957, 1961) started the process with a number of groups in tropical Africa but never articulated his reasoning beyond a statement that *Asclepias* should refer only to New World species and that he proposed to “exclude *Asclepias* Linn. from Africa, except as an adventive, and to divide further

the species hitherto assigned to it into other genera” (1952: 406). Some of Bullock’s generic concepts have been developed by later workers—*Aspidoglossum* E. Mey., *Miraglossum* Kupicha, and *Schizoglossum* (Kupicha, 1984); *Aspidonepsis* Nicholas & Goyder (Nicholas & Goyder, 1992); *Glossostelma* (Goyder, 1995); *Gomphocarpus* R. Br. (Goyder, 2001b; Goyder & Nicholas, 2001); *Margaretta* Oliv. (Goyder, 2005); *Pachycarpus* E. Mey. (Smith, 1988; Goyder, 1998a); *Stathmostelma* K. Schum. (Goyder, 1998b); and *Trachycalymma* (K. Schum.) Bullock (Goyder, 2001a). In southern Africa, Bullock’s unpublished views on *Xysmalobium* sensu N. E. Br. (1902), as deduced from annotations of herbarium collections housed at K, formed the basis of a postgraduate thesis (Langley, 1980), but the generic changes proposed therein were never formally published. Likewise, unpublished theses by Nicholas (1982, 1999) provide a framework for the *Asclepias* complex in southern Africa, but a satisfactory synthesis of tropical and southern African taxa is still lacking. The present contribution is designed firstly to test the monophyly of the *Asclepias* generic complex, and secondly to see what lineages can be detected within this assemblage.

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MATERIAL AND METHODS

TAXA

Sixty-five species in 21 out of the 24 recognized Asclepiadinae genera and one undescribed genus constitute the ingroup (Table 1). Two accessions from different localities were analyzed in each of five species (*Asclepias crispa* P. J. Bergius, *A. gibba* (E. Mey.) Schltr., *Aspidoglossum heterophyllum* E. Mey., *Gomphocarpus physocarpus*, and *Kanahia laniflora* (Forssk.) R. Br.). In *Asclepias*, the only genus represented in both the Old World and the New World, 10 Old World species and six New World species were included. *Marsdenia sylvestris* (Retz.) P. I. Forst. (Marsdenieae) was used as the most distant outgroup. As closer outgroups, species were selected from the closely related subtribes of the Asclepiadeae, the Tylophorinae, and Cynanchinae, including *Glossonema* Decne. and *Odontanthera* Wight. Exemplars from the American Metastelminae–Gonolobinae–Oxy-petalinae assemblage, sister to the Asclepiadinae–Cynanchinae–Tylophorinae clade (Rapini et al., 2003; Liede-Schumann et al., 2005; Goyder, 2006), were not included, because studies by Liede (2001) and Liede and Täuber (2002) had shown that sequences from this taxonomic group added numerous long indels but presented little additional information as to the directionality of site changes. Instead, taxa previously found not to fit in any of the recognized subtribes were included: *Eustegia minuta* (L. f.) N. E. Br. (Rapini et al., 2003); *Calciophila galgalensis* (Liede) Liede & Meve (Liede & Kunze, 2002; Liede-Schumann & Meve, 2006); *Oxystelma bornouense* R. Br. and *O. esculentum* (L. f.) Schult. (Liede & Täuber, 2000); and *Solenostemma arghel* (Delile) Hayne (Liede et al., 2002a—as *S. oleifolium* (Nectoux) Bullock & E. A. Bruce).

DNA EXTRACTION AND PCR

DNA was isolated from fresh or dried leaf tissue using the CTAB protocols (Doyle & Doyle, 1987). Polymerase chain reaction (PCR) primers and protocol for the plastid *trnT-L* and *trnL-F* spacers (primers “a,” “b” and “e,” “f,” respectively) and the *trnL* intron (primers “c” and “d”) correspond exactly to Taberlet et al. (1991). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer (Applied Biosystems, Foster City, California, U.S.A.). Of the 80 accessions, 65 have been sequenced for this study; the remaining 15 were previously deposited at European Molecular Biology Laboratory (EMBL; Table 1). The *trnL* intron and *trnL-F* spacer of *Asclepias syriaca* L. and *A. tuberosa* L. were taken from EMBL, and we were able to supplement the missing partial sequences

(*trnT-L* spacer and ITS) from our own material for *A. syriaca*, but not for *A. tuberosa*.

The entire nuclear internal transcribed spacer region (ITS) including 5.8S of ribosomal DNA (rDNA) was amplified using the flanking primers ITS4 and ITS5 following a modified protocol based on Baldwin (1992) and described in Meve and Liede (2001). ITS sequences of 68 species were obtained for the present study, with one sequence studied previously. An interpretable ITS sequence could not be obtained for *Trachycalymma pseudofimbriatum* Goyder. Published or unpublished ITS sequences by the last author are available for all outgroup taxa except for *Oxystelma bornouense*. However, only those of *O. esculentum* and *Solenostemma arghel* could be aligned to the Asclepiadinae sequences, albeit with difficulties.

DATA ANALYSIS

Sequences were pre-aligned with Sequence Navigator Version 1.0.1 (Perkin-Elmer, Inc., Waltham, Massachusetts, U.S.A.) and manually adjusted. Separate indel coding was not performed, because the number of phylogenetically informative indels was very low in the chloroplast DNA (cpDNA) data set, while the indels could not be coded unambiguously in the ITS data set. Both data sets were analyzed separately and in combination. Phylogenetic analysis and tests for clade support were performed, using PAUP version 4.0b8 (PPC) (Swofford, 1998). Phylogenies were generated using Fitch parsimony as implemented in PAUP* employing heuristic searches, with 1000 replicates, random stepwise addition, MULPARS off, and tree-bisection-reconnection (TBR) branch swapping. The resulting trees were then used as starting trees for a second round of search with MULPARS on. Our search strategy aimed at finding as many different islands of trees as possible. Sets of equally parsimonious trees recovered from each analysis were summarized by strict consensus. Decay indices (Bremer, 1988; Donoghue et al., 1992) and bootstrap values (Felsenstein, 1985) derived from 1000 replicates (maxtrees set to 100) were calculated as measures of support for individual clades. Decay analyses were performed with Auto-Decay 4.0 (Eriksson, 1998) in combination with the reverse constraint option of PAUP*. A partition homogeneity test as implemented in PAUP* was conducted (10 addition sequence replicates) to test the congruence of the cpDNA and the ITS data sets.

RESULTS

The sequence alignment is available from the authors and from TreeBASE (study accession number

Table 1. Voucher specimens and EMBL accession numbers for the taxa investigated. Sequences already published: A = Liede and Täuber (2000); B = Liede and Kunze (2002); C = Liede (2001); D = Liede et al. (2002a); E = Liede et al. (2002b); F = Meve and Liede (2001); and G = Potgieter and Albert (2001). ITS sequence numbers in square brackets refer to published sequences that could not be fitted into the ITS alignment.

Tribe, subtribe	Taxon	DNA voucher	<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	ITS
Marsdenieae	<i>Marsdenia sylvestris</i> (Retz.) P. I. Forst.	Cameroon: Mokolo, <i>Meve 919</i> (UBT)	AJ402118 (F)	AJ402137 (F)	AJ402142 (F)	unpubl.
Asclepiadeae, unplaced	<i>Calciophila galgalensis</i> (Liede) Liede & Meve	Somalia: Bari, <i>Thulin & Warfa 6205</i> (UPS)	AJ492337 (B)	AJ492338 (B)	AJ492339 (B)	[AJ492756 (B)]
	<i>Eustegia minuta</i> (L. f.) N. E. Br.	South Africa: <i>Bruyns 4357</i> (K)	AJ410205 (C)	AJ410206 (C)	AJ410207 (C)	unpubl.
	<i>Oxystelma bornouense</i> R. Br.	Kenya: Tsavo East, <i>Liede & Newton 3210</i> (UBT)	AJ290883 (A)	AJ290882 (A)	AJ290881 (A)	—
	<i>Oxystelma esculentum</i> (L. f.) Schult. ex <i>Shirley s.n.</i> (UBT)	Egypt: Elephantine Island, cult. Bayreuth	AJ290884 (A)	AJ290885 (A)	AJ290887 (A)	AM396877
Asclepiadeae, Cynanchinae	<i>Solenostemma arghel</i> (Delile) Hayne	Egypt: <i>Aziz s.n.</i> (MØ)	AJ428831 (D)	AJ428832 (D)	AJ428833 (D)	AM396878
	<i>Cynanchum ellipticum</i> (Harv.) R. A. Dyer	South Africa: Eastern Cape, <i>Liede 2933</i> (UBT)	AJ290845 (A)	AJ290846 (A)	AJ290847 (A)	[AJ320444 (E)]
	<i>Glossonema boveanum</i> (Decne.) Decne.	Kenya: Loyengalani, <i>Liede & Newton 3239</i> (ULM)	AJ428804 (D)	AJ428805 (D)	AJ428806 (D)	[AJ492803 (B)]
	<i>Odontanthera radians</i> (Forssk.) D. V. Field	Yemen: North, <i>Müller-Hohenstein & Deil 1967</i> (UBT)	AJ428813 (D)	AJ428814 (D)	AJ428815 (D)	[AJ492809 (B)]
Asclepiadeae, Tylophorinae	<i>Tylophora flexuosa</i> R. Br.	Philippines: Laguna, <i>Liede 3252</i> (UBT)	AJ290915 (A)	AJ290916 (A)	AJ190917 (A)	[AJ320460 (E)]
Asclepiadeae, Asclepiadinae	<i>Asclepias albens</i> (E. Mey.) Schltr.	South Africa: Eastern Cape, <i>Nicholas 2813</i> (UDW)	AM295719	AM295720	AM295721	AM396879
	<i>Asclepias brevipes</i> (Schltr.) Schltr.	South Africa: Free State, <i>Nicholas 2866</i> (UDW)	AM295724	AM295723	AM295722	AM396880
	<i>Asclepias crispa</i> P. J. Bergius, acc. 1	South Africa: Eastern Cape, <i>Nicholas 2814</i> (UDW)	AM295725	AM295726	AM295727	AM396881
	<i>Asclepias crispa</i> P. J. Bergius, acc. 2	South Africa: Eastern Cape, <i>Nicholas 2823</i> (UDW)	AM295730	AM295729	AM295728	AM396882
	<i>Asclepias cucullata</i> (Schltr.) Schltr.	South Africa: KwaZulu-Natal, <i>Nicholas 2842</i> (UDW)	AM295731	AM295732	AM295733	AM396883
	<i>Asclepias curassavica</i> L., acc. 1	Venezuela: Mérida, <i>Liede & Meve 3316</i> (UBT)	AM295734	—	—	AM396884
	<i>Asclepias curassavica</i> L., acc. 2	U.S.A.: <i>Potgieter & Cahilly 243</i> (NY)	—	AF102381 (G)	AF214156 (G)	—
	<i>Asclepias fascicularis</i> Decne.	U.S.A.: ex hort UC Davis	AM295735	AM295736	AM295737	AM396885

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	ITS
	<i>Asclepias gibba</i> (E. Mey.) Schltr., acc. 1	South Africa: Eastern Cape, <i>Nicholas 2824</i> (UDW)	AM295740	AM295739	AM295738	AM396886
	<i>Asclepias gibba</i> (E. Mey.) Schltr., acc. 2	South Africa: Eastern Cape, <i>Nicholas 2838</i> (UDW)	AM295741	AM295742	AM295743	AM396887
	<i>Asclepias linaria</i> Cav.	Mexico: Michoacán, <i>Conrad 9305</i> (UBT)	AM295746	AM295745	AM295744	AM396888
	<i>Asclepias longifolia</i> Michx.	U.S.A.: <i>Wyatt s.n.</i> (UGA)	AM295747	AM295748	AM295749	AM396889
	<i>Asclepias praemorsa</i> Schltr.	South Africa: Transkei, <i>Ollerton 214</i> (NU, spirit)	AM295718	AM295717	AM295716	AM396890
	<i>Asclepias stellifera</i> Schltr.	South Africa: KwaZulu-Natal, <i>Nicholas 2841</i> (UDW)	AM295713	AM295714	AM295715	AM396891
	<i>Asclepias syriaca</i> L.	ex hort. Münster, in cult. Münster (MSUN)	AJ410178 (C)	AJ410179 (C)	AJ410180 (C)	AM396892
	<i>Asclepias tuberosa</i> L.	U.S.A.: <i>Potgieter & Cahilly 242</i> (NY)	—	AF214312 (G)	AF141158 (G)	—
	<i>Asclepias woodii</i> (Schltr.) Schltr.	South Africa: KwaZulu-Natal, <i>Ollerton 255</i> (NU, spirit)	AM295712	AM295711	AM295710	AM396893
	<i>Aspidoglossum connatum</i> (N. E. Br.) Bullock	Tanzania: Nkansi District, <i>Bidgood et al. 2583</i> (K)	AM295700	AM295699	AM295698	AM396894
	<i>Aspidoglossum delagoense</i> (Schltr.) Kupicha	South Africa: KwaZulu-Natal, <i>Nicholas 2877</i> (UDW)	AM295707	AM295708	AM295709	AM396895
	<i>Aspidoglossum heterophyllum</i> E. Mey., acc. 1	South Africa: Western Cape, <i>Bruyns 6855</i> (K)	AM295701	AM295702	AM295703	AM396896
	<i>Aspidoglossum heterophyllum</i> E. Mey., acc. 2	South Africa: Eastern Cape, <i>Nicholas 2816</i> (UDW)	AM295706	AM295705	AM295704	AM396897
	<i>Aspidonepsis diploglossa</i> (Turcz.) Nicholas & Goyder	South Africa: KwaZulu-Natal, <i>Ollerton 228</i> (NU, spirit)	AM295695	AM295696	AM295697	AM396898
	<i>Aspidonepsis flava</i> (N. E. Br.) Nicholas & Goyder	South Africa: KwaZulu-Natal, <i>Ollerton 223</i> (NU, spirit)	AM295694	AM295693	AM295692	AM396899
	<i>Calotropis procera</i> (Aiton) W. T. Aiton	Gambia: <i>Huber s.n.</i> (UBT)	AJ428795 (D)	AJ428796 (D)	AJ428797 (D)	AM396900
	<i>Cordylogyne globosa</i> E. Mey.	South Africa: Mpumalanga, <i>Nicholas 2867</i> (UDW)	AM295691	AM295690	AM295689	AM396901
	<i>Fanninia caloglossa</i> Harv.	South Africa: <i>Ollerton 217</i> (NU, in spirit)	AM295686	AM295687	AM295688	AM396902
	<i>Glossostelma ceciliae</i> (N. E. Br.) Goyder	Tanzania: Ufipa District, <i>Goyder et al. 3834</i> (K)	AM295685	AM295769	AM295768	AM396903

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	ITS
	<i>Gomphocarpus abyssinicus</i> Decne.	Ethiopia: Shewa Region, <i>Liede & Meve</i> 3520 (UBT)	AM295752	AM295751	AM295750	AM396904
	<i>Gomphocarpus cancellatus</i> (Burm. f.) Bruyns	South Africa: <i>Drewe</i> 534 (K)	AM295765	AM295766	AM295767	AM396905
	<i>Gomphocarpus fruticosus</i> (L.) W. T. Aiton	South Africa: Eastern Cape, <i>Nicholas</i> 2796 (UDW)	AM295764	AM295763	AM295762	AM396906
	<i>Gomphocarpus glaucophyllus</i> Schltr.	Zambia: Lusaka, <i>Strid</i> 2266 (K)	AM295759	AM295760	AM295761	AM396907
	<i>Gomphocarpus physocarpus</i> E. Mey., acc. 1	South Africa: Eastern Cape, <i>Nicholas</i> 2798 (UDW)	AM295758	AM295757	AM295756	AM396908
	<i>Gomphocarpus physocarpus</i> E. Mey., acc. 2	South Africa: Eastern Cape, <i>Nicholas</i> 2829 (NH)	AJ290875 (A)	AJ290876 (A)	AJ290877 (A)	AJ320446 (E)
	<i>Gomphocarpus tomentosus</i> Burch.	South Africa: KwaZulu-Natal, <i>Nicholas</i> 2878 (UDW)	AM295753	AM295754	AM295755	AM396872
	<i>Kanahia laniflora</i> (Forssk.) R. Br., acc. 1	Kenya: Tsavo East, <i>Liede & Newton</i> 3211 (ULM)	AM295577	AM295578	AM295579	AM396873
	<i>Kanahia laniflora</i> (Forssk.) R. Br., acc. 2	Yemen: <i>Mangelsdorff</i> 116 (UBT)	AM295582	AM295581	AM295580	AM396874
	<i>Lagarinthus multicaulis</i> E. Mey.	South Africa: Eastern Cape, <i>Nicholas</i> 2836 (UDW)	AM295583	AM295584	AM295585	AM396875
	<i>Margaretta rosea</i> Oliv. subsp. <i>corallina</i> Goyder	Tanzania: Ufipa District, <i>Goyder et al.</i> 3791 (K)	AM295588	AM295587	AM295586	AM396876
	<i>Miraglossum verticillare</i> (Schltr.) Kupicha	South Africa: KwaZulu-Natal, <i>Ollerton</i> 244 (NU, spirit)	AM295589	AM295590	AM295591	AM396840
	<i>Pachycarpus appendiculatus</i> E. Mey.	South Africa: KwaZulu-Natal, <i>Nicholas</i> 2871 (UDW)	AM295594	AM295593	AM295592	AM396841
	<i>Pachycarpus asperifolius</i> Meisn.	South Africa: Limpopo, <i>Nicholas</i> 2858 (UDW)	AM295595	AM295596	AM295597	AM396842
	<i>Pachycarpus concolor</i> E. Mey.	South Africa: KwaZulu-Natal, <i>Nicholas</i> 2856 (UDW)	AM295600	AM295599	AM295598	AM396843
	<i>Pachycarpus coronarius</i> E. Mey.	South Africa: Eastern Cape, <i>Nicholas</i> 2812 (UDW)	AM295601	AM295602	AM295603	AM396844
	<i>Pachycarpus dealbatus</i> E. Mey.	South Africa: KwaZulu-Natal, <i>Nicholas</i> 2849 (UDW)	AM295606	AM295605	AM295604	AM396845
	<i>Pachycarpus goetzei</i> (K. Schum.) Bullock	Tanzania: Njombe District, <i>Bidgood et al.</i> 2106 (K)	AM295607	AM295608	AM295609	AM396846

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	<i>trnT</i> - <i>L</i> spacer	<i>trnL</i> intron	<i>trnL</i> - <i>F</i> spacer	ITS
	<i>Pachycarpus grantii</i> (Oliv.) Bullock subsp. <i>marroninus</i> Goyder	Tanzania: Njombe District, <i>Goyder et al.</i> 3886 (K)	AM295612	AM295611	AM295610	AM396847
	<i>Pachycarpus lineolatus</i> (Decne.) Bullock	Tanzania: Ufipa District, <i>Goyder et al.</i> 3779 (K)	AM295613	AM295614	AM295615	AM396848
	<i>Pachycarpus natalensis</i> N. E. Br.	South Africa: KwaZulu-Natal, <i>Ollerton</i> 254 (NU, spirit)	AM295618	AM295617	AM295616	AM396849
	<i>Pachycarpus reflectens</i> E. Mey.	South Africa: Eastern Cape, <i>Nicholas</i> 2801 (UDW)	AM295619	AM295620	AM295621	AM396850
	<i>Pergularia daemia</i> (Forssk.) Chiov.	Tanzania: Arusha, <i>Masinde</i> 888 (in cult. Bayreuth)	AJ290891 (A)	AJ290892 (A)	AJ290893 (A)	AM396851
	<i>Schizoglossum atropurpureum</i> E. Mey.	South Africa: Eastern Cape, <i>Ollerton</i> 216 (NU, spirit)	AM295624	AM295623	AM295622	AM396852
	<i>Schizoglossum cordifolium</i> E. Mey.	South Africa: <i>Ward s.n.</i> (UDW)	AM295625	AM295626	AM295627	AM396853
	<i>Schizoglossum eustegioides</i> (E. Mey.) Druce	South Africa: North Western Province, <i>Nicholas</i> 2865 (UDW)	AM295630	AM295629	AM295628	AM396854
	<i>Schizoglossum filiforme</i> (L. f.) Druce	South Africa: KwaZulu-Natal, <i>Nicholas</i> 2850 (UDW)	AM295631	AM295632	AM295633	AM396855
	<i>Stathmostelma diversifolium</i> Goyder	Kenya: Narok, <i>Liede & Newton</i> 3221 (ULM)	AM295636	AM295635	AM295634	AM396856
	<i>Stathmostelma pauciflorum</i> (Klotzsch) K. Schum.	Zimbabwe: Karoi, <i>Nicholas</i> 2860 (UDW)	AM295637	AM295638	AM295639	AM396857
	<i>Stathmostelma verdickii</i> De Wild.	Tanzania: Ufipa District, <i>Bidgood et al.</i> 2647 (K)	AM295642	AM295641	AM295640	AM396858
	<i>Stenostelma corniculatum</i> (E. Mey.) Bullock	South Africa: Mpumalanga, <i>Balkwill</i> 10908 (K)	AM295643	AM295644	AM295645	AM396859
	<i>Trachycalymma buchwaldii</i> (Schltr. & K. Schum.) Goyder	Tanzania: Iringa Distr., <i>Goyder et al.</i> 3924 (K)	AM295648	AM295647	AM295646	AM396860
	<i>Trachycalymma foliosum</i> (K. Schum.) Goyder	Tanzania: Ufipa District, <i>Goldblatt et al.</i> 8100 (K)	AM295684	AM295649	AM295650	AM396861
	<i>Trachycalymma pseudofimbriatum</i> Goyder	Ethiopia: Sidamo, <i>Haugen</i> 1503 (K)	AM295653	AM295652	AM295651	—
	<i>Woodia mucronata</i> (Thunb.) N. E. Br.	South Africa: Eastern Cape, <i>Nicholas</i> 2809 (UDW)	AM295654	AM295655	AM295656	AM396862
	<i>Xysmalobium fraternum</i> N. E. Br.	Tanzania: Sumbawanga District, <i>Bidgood</i> <i>et al.</i> 2390 (K)	AM295659	AM295658	AM295657	AM396863

Table 1. Continued.

Tribe, subtribe	Taxon	DNA voucher	<i>trnT-L</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	ITS
	<i>Xysmalobium gerrardii</i> Scott-Elliott	South Africa: KwaZulu-Natal, Ollerton 251 (NU, spirit)	AM295660	AM295661	AM295662	AM396864
	<i>Xysmalobium heudelotianum</i> Decne.	Tanzania: Ufipa District, Goyder et al. 3830 (K)	AM295665	AM295664	AM295663	AM396865
	<i>Xysmalobium involucratum</i> (E. Mey.) Decne.	South Africa: Eastern Cape, Nicholas 2802 (UDW)	AM295666	AM295667	AM295668	AM396866
	<i>Xysmalobium kaessneri</i> S. Moore	Tanzania: Mbeya District, Lovett et al. 3980 (K)	AM295671	AM295670	AM295669	AM396867
	<i>Xysmalobium parviflorum</i> Harv. ex Scott-Elliott	South Africa: KwaZulu-Natal, Nicholas 2840 (UDW)	AM295672	AM295673	AM295674	AM396868
	<i>Xysmalobium tysonianum</i> (Schltr.) N. E. Br.	South Africa: KwaZulu-Natal, Ollerton 233 (NU, spirit)	AM295677	AM295676	AM295675	AM396869
	<i>Xysmalobium undulatum</i> (L.) W. T. Aiton	South Africa: Eastern Cape, Nicholas 2830 (UDW)	AM295678	AM295679	AM295680	AM396870
	Gen. indet., aff. <i>Asclepias</i>	Tanzania: Njombe District, Goyder et al. 3895 (K)	AM295683	AM295682	AM295681	AM396871

Table 2. Summary statistics of the data sets and of the trees resulting from parsimony analysis. CI = consistency index; RI = retention index; RC = rescaled consistency index.

	cpDNA	ITS	Combined
Parsimony informative characters	153	208	277
Unknown data cells	222 (<i>trnT-L</i> spacer) 50 (<i>trnL</i> intron) 131 (<i>trnL-F</i> spacer)	28	
Missing (partial) sequences	<i>Asclepias tuberosa</i> (<i>trnT-L</i> spacer)	<i>Asclepias tuberosa</i> <i>Trachycalymma pseudofimbriatum</i> <i>Oxystelma esculentum</i>	
Outgroup	<i>Marsdenia sylvestris</i> <i>Eustegia minuta</i> <i>Calciophila galgalensis</i> <i>Cynanchum ellipticum</i> <i>Glossonema boreanum</i> <i>Odontanthera radians</i> <i>Tylophora flexuosa</i> <i>Oxystelma bornouense</i> <i>Oxystelma esculentum</i> <i>Solenostemma arghel</i>	<i>Oxystelma esculentum</i> <i>Solenostemma arghel</i>	<i>Oxystelma esculentum</i> <i>Solenostemma arghel</i>
No. of trees (limited by computer capacity)	56,100	56,500	62,000
No. of steps	262	650	778
CI	0.71	0.5	0.52
RI	0.81	0.5	0.66
RC	0.57	0.32	0.34

= S1600, matrix accession numbers = M2879 (cpDNA), M2880 (ITS), M2881 (combined); Sander-son et al., 1994) and comprises 80 taxa and 2847 characters (1088 characters in the *trnT-L* spacer (primers a–b), 530 characters in the *trnL*-intron (primers c–d), 429 characters in the *trnL-F* spacer (primers e–f), and 800 characters for ITS). Details of the data sets, as well as statistics of the trees resulting from parsimony analysis, are listed in Table 2.

The partition homogeneity test shows no significant discordance between the cpDNA and the ITS data sets ($P = 0.09$), and the retrieved clade structure in the cpDNA data set agrees largely with the one retrieved from the ITS data set, despite the poor resolution of the former.

The analyses reveal that the Asclepiadinae as presently circumscribed are monophyletic. The small Old World genera *Pergularia* L. (*P. daemia* (Forssk.) Chiov.), *Calotropis* R. Br. (*C. procera* (Aiton) W. T. Aiton), and *Kanahia* R. Br. (*K. laniflora* (Forssk.) R. Br.) are sister to the remaining members of the subtribe, which themselves form a well-supported clade, referred to here as the *Asclepias* generic complex. Several smaller clades were detected, but overall resolution is limited. The genera in this complex will need to be recircumscribed following our results.

DISCUSSION

The core group of principally herbaceous genera around *Asclepias* contains most of the species diversity within the Asclepiadinae. This group is both mono-phyletic and well supported, resolving into two clades in the ITS (not shown) and combined analyses (Fig. 1). One clade consists of taxa found only in the New World (*A. linaria* Cav., *A. curassavica* L., *A. fascicularis* Decne., *A. longifolia* Michx., and *A. syriaca* L.); the other consists solely of Old World taxa. The *trnT-L* data set reveals no overall resolution into New or Old World lineages, but several minor Old World clades and one minor New World clade are retrieved (not shown). *Asclepias*, the oldest generic name in the complex (1753), must be applied either to the whole assemblage or, if other genera are recognized, to just the New World clade, as this contains *A. syriaca*, its type species. This result is consistent with Bullock’s (1952) assertion that *Asclepias* should be restricted to New World taxa. Within the African radiation, a number of clades were detected, which are well supported in one or more of the analyses.

A clade comprising *Xysmalobium heudelotianum* Decne., *X. fraternum* N. E. Br., *X. kaessneri* S. Moore, *Stathmostelma verdickii* De Wild. (a species excluded

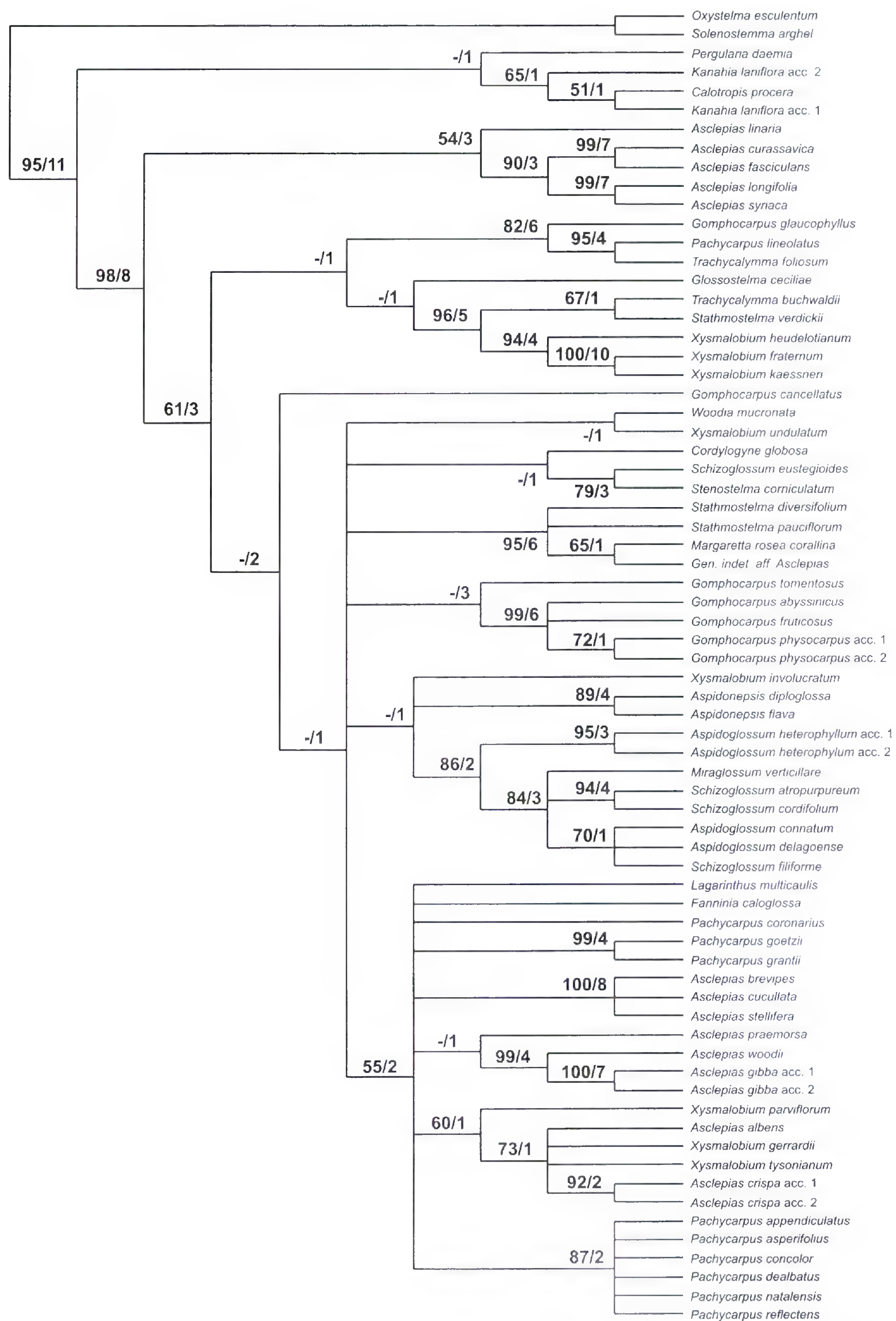


Figure 1. Strict consensus of 62,000 trees resulting from analysis of the combined data set (I = 778 steps, excluding uninformative characters, CI = 0.52, excluding uninformative characters, RI = 0.66, RC = 0.34). Numbers mapped on the tree indicate bootstrap percentages above 50% and decay values.

from *Stathmostelma* by Goyder (1998b) who suggested an affinity with *Gomphocarpus longissimus* K. Schum.), and *Trachycalymma buchwaldii* (Schltr. & K. Schum.) Goyder was detected in all analyses. These species are found in tropical African wooded savannas at moderate to high altitude. A second group of *Xysmalobium* species (*X. parviflorum* Harv. ex Scott-Elliott, *X. gerrardii* Scott-Elliott, and *X. tysonianum* (Schltr.) N. E. Br.) forms a clade with a group of broad-leaved *Asclepias* (*A. albens* (E. Mey.) Schltr. and *A. crispa*) with moderate support (60%) in all analyses. These species share a similar habit with many decumbent stems arising from a large woody tuber and have dense umbelliform pedunculate inflorescences. They occur in open grassland habitats in southeastern parts of tropical and subtropical Africa. The position of the apparently closely allied taxon, *Lagarinthus multicaulis* E. Mey. (Langley, 1980; = *A. multicaulis* (E. Mey.) Schltr. non *A. multicaulis* Vell.), is unresolved, however. *Xysmalobium*, clearly, is polyphyletic as currently circumscribed (Langley, 1980), and the type of the genus, *X. undulatum* (L.) W. T. Aiton, occurs in yet another clade separate from other exemplars of the genus.

Asclepias woodii (Schltr.) Schltr. links with *A. gibba* in ITS and the combined analyses with strong support (99%). This is surprising, because they do not share obvious similarities of habit and vegetative or floral morphology (Brown, 1908). *Asclepias gibba* is much branched from the base, while *A. woodii* is an erect, single-stemmed herb; the corona of *A. gibba* terminates in a long drawn-out apical tooth much taller than the staminal column, while that of *A. woodii* is shorter and lacks the terminal appendage.

The *Schizoglossum*/*Aspidoglossum*/*Miraglossum* complex of Kupicha (1984) is identified in all analyses (*A. heterophyllum*, *A. connatum* (N. E. Br.) Bullock, *A. delagoense* (Schltr.) Kupicha, *M. verticillare* (Schltr.) Kupicha, *S. atropurpureum* E. Mey., *S. cordifolium* E. Mey., and *S. filiforme* (L. f.) Druce; bootstrap 86% in the combined analysis). Kupicha (1984) noted the similarities of *S. filiforme* to species of *Aspidoglossum* but excluded it from the latter genus because of its pedunculate inflorescences and differing corona morphology. The results here link it to *A. connatum* and *A. delagoense*. *Schizoglossum eustegioides* (E. Mey.) Druce was excluded from the complex altogether by Kupicha (1984), who suggested its affinities might lie with *Stenostelma* Schltr.; ITS and the combined analyses support this suggestion, but there is no resolution with *trnT-L*.

A small group of narrow-leaved *Asclepias* species in southern Africa—*A. brevipes* (Schltr.) Schltr., *A. cucullata* (Schltr.) Schltr., and *A. stellifera* Schltr.—occurs in all analyses.

A group of short-lived *Gomphocarpus* species with narrow leaves (*G. abyssinicus* Decne., *G. fruticosus* (L.) W. T. Aiton, and *G. physocarpus* E. Mey.) forms a well-supported clade (bootstrap 99%) with ITS and the combined analyses but is not resolved with *trnT-L*. Neither *G. cancellatus* (Burm. f.) Bruyns nor *G. glaucophyllus* Schltr., both broad-leaved species, form part of this core *Gomphocarpus* lineage. *Gomphocarpus cancellatus* is the only species of the generic complex with a predominantly winter-rainfall distribution in southern Africa and has some anomalous morphological features, such as the combination of broad leaves with perennial rather than annual stems and the more robust processes on the follicle. *Gomphocarpus glaucophyllus* appears in a predominantly tropical clade sister to *Pachycarpus lineolatus* (Decne.) Bullock and *Trachycalymma foliosum* (K. Schum.) Goyder. *Gomphocarpus glaucophyllus* is found in burnt grasslands and savanna woodlands of south tropical Africa, and its broad glaucous leaves are borne on annual shoots from a perennial woody rootstock. In contrast, the narrow-leaved species of *Gomphocarpus* occur in open disturbed habitats in temperate regions of southern Africa and are shrubby in habit, but are probably short-lived and generally lack thickened or tuberous roots.

Stathmostelma diversifolium Goyder, *S. pauciflorum* (Klotzsch) K. Schum., *Margaretta rosea* Oliv. subsp. *corallina* Goyder, and the undescribed taxon, all from seasonally burned montane habitats in tropical Africa, also form a well-supported clade with ITS and the combined analysis but are unresolved with the chloroplast data set. Goyder (2005) pointed to similarities in corona morphology between *Margaretta* and *Stathmostelma*. The undescribed taxon is one of several from the highlands of southern Tanzania and northern Malawi that the first author has found difficult to assign to the genera currently recognized.

Finally, a group of southern African *Pachycarpus* species (*P. appendiculatus* E. Mey., *P. asperifolius* Meissn., *P. concolor* E. Mey., *P. dealbatus* E. Mey., *P. natalensis* N. E. Br., and *P. reflectens* E. Mey.) is detected in *trnT-L* and the combined analyses. Two of these (*P. concolor* and *P. dealbatus*) form a minor clade in ITS, as do two of the tropical species (*P. goetzei* (K. Schum.) Bullock and *P. grantii* (Oliv.) Bullock subsp. *marroninus* Goyder). However, *P. coronarius* E. Mey. remains in an unresolved position in the combined analysis, and *P. lineolatus*, a widely distributed tropical species, links somewhat unexpectedly to the south tropical African species *Gomphocarpus glaucophyllus*, as already indicated.

In the exemplars above, vegetative features seem a more reliable guide to evolutionary relationships than do the traditional floral characters. In particular,

there has been a switch between the *Xysmalobium*-type fleshy corona and *Asclepias*- or *Gomphocarpus*-type cucullate corona both in tropical and southern African clades. *Margaretta* and *Stathmostelma* sensu Goyder (1998b) have much more showy and brightly colored flowers than the small white flowers of the undescribed taxon but are similar vegetatively with erect annual stems arising from a carrot-shaped tuber.

The delimitation of *Asclepias* could be restricted to species from the Americas, and the Old World clade subdivided into genera with redefined limits. This would require a more intensively sampled survey and the use of additional genomes. Some of these lineages might correspond to the tropical group with erect, fusiform follicles (e.g., *Xysmalobium heudelotianum*); a more narrowly defined *Gomphocarpus*; an expanded *Margaretta* containing *Stathmostelma* and some tropical taxa currently unassigned to genus; *Schizoglossum* including *Miraglossum* and *Aspidoglossum* but excluding some anomalous taxa; and the broad-leaved group of *Xysmalobium* and *Asclepias* from southern African grasslands.

Literature Cited

- Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molec. Phylogen. Evol.* 1: 3–16.
- Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.
- Brown, N. E. 1902–1903. Asclepiadeae. Pp. 231–503 in W. T. Thiselton-Dyer (editor), *Flora of Tropical Africa*, Vol. 4(1). Lovell Reeve & Co., London.
- . 1908. *Asclepias*. Pp. 663–714 in W. T. Thiselton-Dyer (editor), *Flora Capensis*, Vol. 4(1). Lovell Reeve & Co., London.
- Bullock, A. A. 1952. Notes on African Asclepiadaceae. I. *Kew Bull.* 7: 405–426.
- . 1953a. Notes on African Asclepiadaceae. II. *Kew Bull.* 8: 51–67.
- . 1953b. Notes on African Asclepiadaceae. III. *Kew Bull.* 8: 329–362.
- . 1955. Notes on African Asclepiadaceae. V. *Kew Bull.* 9: 579–594.
- . 1956. Notes on African Asclepiadaceae. VII. *Kew Bull.* 10: 611–626.
- . 1957. Notes on African Asclepiadaceae. VIII. *Kew Bull.* 11: 503–522.
- . 1961. Notes on African Asclepiadaceae. IX. *Kew Bull.* 15: 193–206.
- Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. *Ann. Missouri Bot. Gard.* 79: 333–345.
- Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Amer.* 19: 11–15.
- Eriksson, T. 1998. AutoDecay 4.0. Stockholm Univ., Stockholm.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- Goyder, D. J. 1995. Notes on the African genus *Glossostelma* (Asclepiadaceae). *Kew Bull.* 50: 527–555.
- . 1998a. A revision of *Pachycarpus* E. Mey. (Asclepiadaceae: Asclepiadeae) in tropical Africa with notes on the genus in southern Africa. *Kew Bull.* 53: 335–374.
- . 1998b. A revision of the African genus *Stathmostelma* K. Schum. (Apocynaceae: Asclepiadeae). *Kew Bull.* 53: 577–616.
- . 2001a. A revision of the tropical African genus *Trachycalymma* (K. Schum.) Bullock (Apocynaceae: Asclepiadoideae). *Kew Bull.* 56: 129–161.
- . 2001b. *Gomphocarpus* (Apocynaceae: Asclepiadeae) in an African and a global context—An outline of the problem. *Biol. Skr.* 54: 55–62.
- . 2005. Intraspecific variation in *Margaretta rosea* Oliv. (Apocynaceae: Asclepiadoideae). *Kew Bull.* 60: 87–94.
- . 2006. An overview of Asclepiad biogeography. Pp. 205–214 in S. A. Ghazanfar & H. J. Beentje (editors), *Taxonomy and Ecology of African Plants, Their Conservation and Sustainable Use*. Royal Botanic Gardens, Kew.
- & A. Nicholas. 2001. A revision of *Gomphocarpus* R. Br. (Apocynaceae: Asclepiadeae). *Kew Bull.* 56: 769–836.
- Kupicha, F. K. 1984. Studies on African Asclepiadaceae. *Kew Bull.* 38: 599–672.
- Langley, R. W. 1980. Taxonomic Studies in the Asclepiadeae with Particular Reference to *Xysmalobium* R. Br. in Southern Africa. M.Sc. Thesis, University of Natal, Pietermaritzburg.
- Liede, S. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae)—A synopsis. *Taxon* 46: 233–247.
- . 2001. Subtribe Astephaninae (Apocynaceae—Asclepiadoideae) reconsidered: New evidence based on cpDNA spacers. *Ann. Missouri Bot. Gard.* 88: 657–668.
- & H. Kunze. 2002. *Cynanchum* and the Cynanchinae (Apocynaceae—Asclepiadoideae): A molecular, anatomical and latex triterpenoid study. *Org. Divers. Evol.* 2: 239–269.
- & A. Täuber. 2000. *Sarcostemma* R. Br. (Apocynaceae—Asclepiadoideae)—A controversial generic circumscription reconsidered: Evidence from *trnL-F* spacers. *Pl. Syst. Evol.* 225: 133–140.
- & ———. 2002. Circumscription of the genus *Cynanchum* (Apocynaceae—Asclepiadoideae). *Syst. Bot.* 27: 789–800.
- , U. Meve & A. Täuber. 2002a. What is subtribe Glossonematiniae (Apocynaceae: Asclepiadoideae)? A phylogenetic study based on cpDNA spacer. *Bot. J. Linn. Soc.* 139: 145–158.
- , A. Täuber & J. Schneidt. 2002b. Molecular considerations on the Tylophorinae K. Schum. (Apocynaceae—Asclepiadoideae). *Edinburgh J. Bot.* 59: 377–403.
- Liede-Schumann, S. & U. Meve. 2006. *Calciophila*, a new genus in African Asclepiadeae (Apocynaceae, Asclepiadoideae), and taxonomic rectifications in *Cynanchum*. *Novon* 16: 368–373.
- , A. Rapini, D. J. Goyder & M. W. Chase. 2005. Phylogenetics of the New World subtribes of Asclepiadeae (Apocynaceae—Asclepiadoideae): Metastelmatinae, Oxypetalinae and Gonolobinae. *Syst. Bot.* 30: 184–195.

- Meve, U. & S. Liede. 2001. Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae–Ceropegieae). *S. Afr. J. Bot.* 67: 161–168.
- Nicholas, A. 1982. Taxonomic Studies in *Asclepias* L. with Particular Reference to the Narrow-leaved Species in Southern Africa. M.Sc. Thesis, University of Natal, Pietermaritzburg.
- . 1999. A Taxonomic Reassessment of the Subtribe Asclepiadinae (Asclepiadaceae) in Southern Africa. Vols. 1 & 2. Ph.D. Thesis, University of Durban-Westville, Durban.
- & D. J. Goyder. 1992. *Aspidonepsis* (Asclepiadaceae), a new southern African genus. *Bothalia* 22: 23–35.
- Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trnL* intron and *trnL-F* spacer sequences and propagule characters. *Ann. Missouri Bot. Gard.* 88: 523–549.
- Rapini, A., M. W. Chase, D. J. Goyder & J. Griffiths. 2003. Asclepiadeae classification: Evaluating the phylogenetic relationships of New World Asclepiadoideae (Apocynaceae). *Taxon* 52: 33–50.
- Sanderson, M. J., M. J. Donoghue, W. Piel & T. Eriksson. 1994. TreeBASE: A prototype database of phylogenetic analyses and an interactive tool for browsing the phylogeny of life. *Amer. J. Bot.* 81: 183.
- Smith, D. M. N. 1988. A revision of the genus *Pachycarpus* in southern Africa. *S. Afr. J. Bot.* 54: 399–439.
- Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), Version 4. Sinauer, Sunderland, Massachusetts.
- Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.